Product Catalog

Enzo

Contents

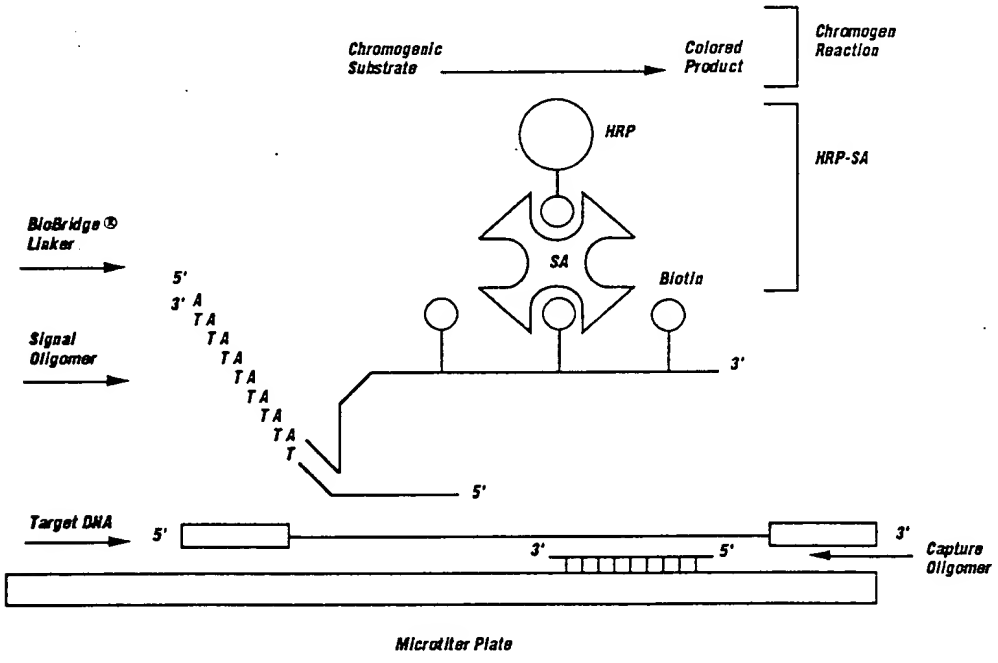
The Enzo Microplate Hybridization Assays*

- **For direct detection of HIV**
- **For specific identification of MTB**

The Enzo Microplate Hybridization Assay System is an easy-to-use, rapid and nonradioactive kit for detecting DNA in a microtiter well. The straightforward assay procedure is based on a two probe hybridization method and is carried out entirely in the 96-well microtiter plate or, alternatively, microwell strips. The generation of a color, which can be easily measured, indicates a positive reaction.

No specialized equipment, other than a standard microplate reader, which is present in most medical research labs, is required. In fact, for plus/minus determinations the results can be read by eye. The assay format uses either a microtiter plate, where up to 96 assays (including controls) can be run, or well strips, where 8 assays per strip (including controls) can be run. The assay requires less than three hours.

Schematic Representation of the Enzo Microplate Hybridization Assay*



Procedure for the Enzo Microplate Hybridization Assay

This nonradioactive procedure involves hybridization of target nucleic acid to a well-bound capture probe followed by binding of a biotinylated signaling probe to the captured target. Immobilized target DNA is then visualized by reaction with a biotin-binding signal generating complex of streptavidin (SA) and horseradish peroxidase (HRP). A positive reaction is indicated by generation of color which can be measured by a microplate reader commonly used in the laboratory. Using this format, 5×10^7 to 1×10^8 copies of target sequences can be detected.

The assay utilizes several unique features. The use of probe pairs increases the specificity of the assay. Two independent hybridization events are required to generate a signal. This type

* *U.S. Patent Nos. 4,711,955; 4,994,373 and Patents Pending*

of assay has been found to be insensitive to the presence of cellular components other than the target DNA sequence. Since the assay is nonradioactive, the components are safe and stable, and the assay can be carried out manually or using devices developed for the automated processing of microtiter plate-based assays.

Denature Sample

Incubate sample with denaturation buffer for 15 minutes to denature the target nucleic acid sequences.

Hybridize to Well-Bound Capture Oligomer

After rinsing the wells, add sample to each well containing hybridization buffer and incubate for 90-120 minutes.

Reaction with Signal Oligomer

After the sample DNA is hybridized to the capture probe, signal probe is added and incubated for 15 minutes.

Addition of Linker

After rinsing the wells, add the linker (which supplies the biotin) to each well and incubate for 10 minutes.

Detection

Bind the streptavidin-horseradish peroxidase complex, wash the wells and then add the chromogen/substrate to generate color.

- A positive result appears as a blue color which turns to yellow upon addition of the stop solution.
- Results may be quantified by reading OD at 450.

References

1. Cook, A.F., Vuocolo, E. and Brakel, C.L. (1988) Nuc. Acid Res. 16:4077.
2. Picken, R.N., Plotch, S.J., Wang, Z., Lin, B.C., Donegan, J.J. and Yang, H.L. (1988) Molecular and Cellular Probes 2:111.

Enzo HIV Microplate Hybridization Assay*

Cat. No. 46330/plate ***Using a 96 well microplate***
Cat. No. 46330/strips ***Using 12 8-well strips***

The Enzo HIV Microplate Hybridization Assay Kit provides materials for the colorimetric detection of nucleic acid containing the *gag* region. HIV DNA can be assayed directly if there is sufficient target DNA present, or it can be assayed in procedures employing target amplification.

The Microplate Assay detects HIV proviral DNA with excellent sensitivity; fewer than 10 proviral sequences can be detected. Furthermore, when the assay is performed using a standard curve of HIV DNA copies, quantitative virus measurements can be done. Thus, the Microplate Assay is readily applicable to studies measuring the effect of drug treatments on virus concentration, virus concentration during the course of infection, virus concentration in animal model studies and numerous other studies where virus quantitation is a critical parameter.

Direct and quantitative HIV detection offers a distinct advantage over serological assays. The presence of provirus DNA can be determined independent of the presence or absense of antibodies which appear several months after initial infection. Thus, this technique offers researchers the potential for identifying proviral DNA in instances where HIV positivity cannot be identified adequately by any of the current means available as, for example, in samples from HIV positive individuals who have not seroconverted.

Enzo MTB Microplate Hybridization Assay*

Cat. No. 46340/plate ***Using a 96 well microplate***
Cat. No. 46340/strips ***Using 12 8-well strips***

The Enzo MTB Hybridization Assay Kit provides materials for the identification of *Mycobacterium tuberculosis* complex. The assay detects only the members of the MTB complex, *i.e.*, *M. africanum, M. bovis, M. tuberculosis, M. microti*, but not 25 other species of *Mycobacteria*, including *M. avium, M. intracellulare, M. kansasii, M. chelonae*. Furthermore, 63 other bacterial species were found to be negative, including a variety of respiratory pathogens. MTB complex DNA can be assayed directly if there is sufficient target DNA present, or it can be assayed in procedures employing target amplification or culture.

While the presence of acid fast bacteria in a specimen can be highly suggestive of a mycobacterial infection, it is necessary to identify the species present. The identification of *Mycobacteria* is often more difficult than many other pathogens since the clinically relevant species do not grow rapidly in laboratory culture. Using the Microplate Assay, the researcher can specifically identify members of the MTB complex under conditions which are unrelated to the state of growth of the culture or specimen.

Oligonucleotides

Cat. No. 46331 ***SK 38/SK 39***
Cat. No. 46341 ***MTB 10/MTB 11***

The SK 38/SK 39 oligonucleotide pair is complementary to the HIV *gag* region. The MTB 10/MTB 11 oligonucleotide pair is complementary to sequences specific for the MTB complex.

The oligonucleotide pairs are supplied in a quantity of 2 nanomoles each oligomer.

** U.S. Patent Nos. 4,711,955; 4,994,373 and Patents Pending*

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